compared to Intersol. This was equally observed in all three subjects, suggesting that the behaviour of the accumulation of PLT-derived bioactive substances such as RANTES or sCD40L depends more on PASs used than donors.

The FPA is cleaved from fibrinogen as a result of thrombin activation and was measured in this study as an indicator of initiation of the coagulation cascade. As shown in Fig. 3a, FPA levels during storage were equivalent to those in normal plasma [31], indicating that diluting plasma with PAS per se did not affect the coagulation cascade. A recent report described the correlation between the level of anaphylatoxin C5a in PCs and the occurrence of allergic reactions after PC transfusion [22]. It is thus expected that C3a, another anaphylatoxin, also accumulates in PCs during storage and exerts a pro-inflammatory effect when PCs are transfused. Although the levels of C3a gradually increased during storage in our study, no marked difference was observed among the four PASs.

In conclusion, M-sol as well as SSP+ and Composol effectively preserved the quality of PCs collected by apheresis. These effects seem to depend on magnesium and potassium. The bioactive substances such as RANTES and sCD40L accumulated less in these PASs. PASs for PCs may thus play a role in preventing a portion of non-haemolytic transfusion reactions as well. Parallel comparison using PCs obtained from the same donor suggested that PC quality largely depended on PASs used but not donors.

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Conflict of interest

The authors have no conflict of interest to disclose.

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World Health Organization International Standard to Harmonize Assays for Detection of Hepatitis E Virus RNA

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Nucleic acid amplification technique–based assays are a primary method for the detection of acute hepatitis E virus (HEV) infection, but assay sensitivity can vary widely. To improve interlaboratory results for the detection and quantification of HEV RNA, a candidate World Health Organization (WHO) International Standard (IS) strain was evaluated in a collaborative study involving 23 laboratories from 10 countries. The IS, code number 6329/10, was formulated by using a genotype 3a HEV strain from a blood donation, diluted in pooled human plasma and lyophilized. A Japanese national standard, representing a genotype 3b HEV strain, was prepared and evaluated in parallel. The potencies of the standards were determined by qualitative and quantitative assays. Assay variability was substantially reduced when HEV RNA concentrations were expressed relative to the IS. Thus, WHO has established 6329/10 as the IS for HEV RNA, with a unitage of 250,000 International Units per milliliter.

Hepatitis E virus (HEV) is a nonenveloped, single-stranded RNA virus belonging to the family *Hepeviridae* (*1*,*2*). In developing countries, HEV is a major cause of acute hepatitis, transmitted by the fecal–oral route and associated with contamination of drinking water. In industrialized countries, reports of HEV infection have been uncommon but are being reported more frequently; some cases are imported after travel to HEV-endemic areas, but reports of autochthonous cases are also increasing, and infection with HEV appears to be more prevalent than originally believed (*3*). Prospects for control of HEV infection are encouraged by recent efforts in vaccine development (*4*,*5*).

Four main genotypes of HEV, representing a single serotype, infect humans. Genotype 1 viruses are found mainly in Africa and Asia and genotype 2 in Africa and Central America; it is in these areas that prevention of HEV infection by vaccination would be most beneficial. Genotypes 3 and 4 viruses are generally less pathogenic, although some exceptions have been reported, particularly for genotype 4; these genotypes infect not only humans but also animals such as swine, wild boar, and deer. Although genotype 4 strains have mainly been restricted to parts of Asia, genotype 3 viruses are found widely throughout the world. Zoonotic transmission of HEV genotypes 3 and 4 to humans can occur by consumption of contaminated meat or meat products or by contact with infected animals (*6*,*7*). Shellfish, such as bivalve mollusks, have also been shown to act as reservoirs for HEV (*8*).

An alternate route of transmission of HEV by transfusion of blood components has been reported in Japan (9,10), the United Kingdom (11), and France (*12*,*13*). Studies in Japan (*14*) and the People's Republic of China (*15*) have identified acute HEV infections in blood donors, confirmed by the detection of HEV RNA. Analysis of blood and plasma donors in Europe has identified HEV-infected donors in Germany (*16*–*20*), Sweden (*18*), and England (*21*). Transmission of HEV by solid organ transplantation has also been reported (*22*). Rates of HEV infection may be underreported in some countries, and misdiagnosis of HEV infection also occurs. For example, in some cases of suspected drug-induced liver injury, HEV has been determined

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[2]In memory of Thomas Laue.

as the cause (*23*). In one such recent case, HEV was shown to have been transmitted by blood transfusion (*13*).

Infection with HEV may cause particularly severe illness in pregnant women and in persons who have preexisting liver disease. Chronic infection with HEV genotype 3 is an emerging problem among solid organ transplant recipients and may also occur in persons with HIV and certain hematologic disorders (*24*). In patients with chronic infection, viral loads are monitored to investigate the efficacy of antiviral treatment (*25*,*26*) and effects of reduction of immunosuppressive therapy (*27*).

HEV infection is diagnosed on the basis of detection of specific antibodies (IgM and IgG), but the sensitivity and specificity of these assays is not optimal (*28*–*30*). Analysis of HEV RNA by using nucleic acid amplification techniques (NATs) is also used for diagnosis; this method can identify active infection and help confirm serologic results (*31*). Several NAT assays have been reported for the detection of HEV RNA in serum and plasma or fecal samples: conventional reverse transcription PCR (RT-PCR) and nested protocols (*32*), real-time RT-PCR, and reverse transcription loop-mediated isothermal amplification (*33*). The NATs include generic assays designed for the detection of HEV genotypes 1–4 (*34*,*35*).

In 2009, the World Health Organization (WHO) Expert Committee on Biological Standardization endorsed a proposal by the Paul-Ehrlich-Institut (PEI) to prepare an International Standard (IS) for HEV RNA for use in NAT-based assays. PEI recently completed an initial study that investigated the performance of HEV NAT assays in detection of HEV infection (*36*). In that study, dilution panels of HEV genotype 3 and 4 strains underwent blinded testing in laboratories that had experience in detection of HEV RNA. Results demonstrated wide variations in assay sensitivity (in the order of 100- to 1,000-fold for most assays).

After the initial study, 2 virus strains included in the panel (*36*) were selected for further development of a candidate IS for the WHO, and a candidate Japanese national standard (done in collaboration with the National Institute of Infectious Diseases in Tokyo). These viruses belong to genotype 3, which is widely distributed, and were genotype 3a and 3b strains, which were equally well detected in the initial study. The strains were derived from plasma samples that had sufficient titers of HEV RNA to prepare standards of good potency. An international collaborative study was conducted to establish the respective standards, demonstrate suitability for use, evaluate potency, and assign an internationally agreed-upon unitage.

Methods

Preparation of Materials

The 2 HEV strains selected for the preparation of the candidate WHO IS and candidate Japanese national standard were genotype 3a strain HRC-HE104 and genotype 3b strain JRC-HE3, respectively. The HEV-positive plasma donations were kindly provided by the Japanese Red Cross Society Blood Service Headquarters (Tokyo, Japan). Characterization of the stock virus strains is shown in Table 1.

The samples were tested for IgG/IgM against HEV by using an HEV enzyme immunoassay (Institute of Immunology Co., Ltd., Tokyo, Japan). Full-length sequences of the HEV strains were determined as described (*37*). Phylogenetic analyses were conducted by using MEGA version 5.05 (*38*), and HEV genotype and subgenotype were determined as described (*39*). The nucleotide sequences of HRC-HE104 and JRC-HE3 were deposited into GenBank under accession nos. AB630970 and AB630971, respectively.

The target HEV RNA concentration for the 2 bulk standard preparations was ≈5.5 $\log_{10}$ HEV RNA copies/mL, on the basis of the concentrations determined in the initial study (*36*). The 2 virus strains were negative when tested for hepatitis B virus, hepatitis C virus, and HIV-1/2 by using the Cobas TaqScreen MPX test (Roche Molecular Systems Inc., Branchburg, NJ, USA). The samples were diluted by using pooled citrated plasma (*36*) that had tested negative by NAT for hepatitis B virus, hepatitis C virus, and HIV-1/2, and HEV and was also negative for antibodies against HEV by using the recomWell IgG and IgM enzyme immunoassays (Mikrogen GmbH, Neuried, Germany). The diluted plasma was placed into 4-mL screw-cap glass vials, freeze dried, filled with nitrogen, sealed with rubber stoppers, and stored at –20°C. Stability studies demonstrated no substantial change in HEV RNA concentration after freeze drying or after 10 months of storage at –20°C (the usual temperature), +4°C, and +20 to +26°C, compared with samples stored at ≤–80°C.

Table 1. HEV strains diluted and lyophilized as candidate standards in study to establish a WHO International Standard for HEV RNA NAT-based assays*

Virus strain	HEV RNA, copies/mL	Genotype	GenBank accession no.	IgM/IgG against HEV	Alanine aminotransferase, IU/L
HRC-HE104	1.6×10^7	3a	AB630970	–/–	36
JRC-HE3	2.5×10^7	3b	AB630971	+/–	398

*Strains were provided by the Japanese Red Cross Society Blood Service Headquarters, Tokyo, Japan. HEV, hepatitis E virus; WHO, World Health Organization; NAT, nucleic acid amplification technique.

Study Design

The collaborative study was conducted by 24 laboratories from 10 countries; each laboratory was randomly assigned a code number. The samples analyzed in the study were coded sample 1 and sample 2 (replicates of the candidate WHO IS) and sample 3 and sample 4 (replicates of the candidate Japanese national standard). Samples were shipped to participants at ambient temperature. Participants tested the samples by using the laboratory's routine assays for HEV RNA, in 4 separate assay runs, using fresh vials of each sample for each run. Quantitative assay results falling within the linear range of the assays were reported in copies/mL. For qualitative assays, participants assayed each sample by a series of 1.0-$\log_{10}$ dilution steps to obtain an initial estimate of an endpoint and then, in 3 subsequent runs, assayed 0.5-$\log_{10}$ dilutions around the endpoint determined in the first run.

Statistical Methods

Quantitative Assays

Evaluation of quantitative assays was restricted to dilutions of 0.0 $\log_{10}$ to -2.5 $\log_{10}$, a range over which the assays of most participants produced comparable data. For comparison of laboratories, the replicate results of each laboratory, corrected for the dilution factor, were combined as the arithmetic mean of $\log_{10}$ copies/mL. Furthermore, these estimates were combined to obtain an overall estimation for each sample by means of a mixed linear model, using laboratory and $\log_{10}$ dilution as random factors.

Qualitative Assays

The data from all assays were pooled to give a series of values for number positive/number tested at each dilution. For each participant, these pooled results were evaluated by means of probit analysis to estimate the concentration at which 50% of the samples tested were positive; for assays in which the change from complete negative to complete positive results occurred in $\leq$2 dilution steps, the Spearman-Kaerber method was applied for estimation. The calculated endpoint was used to give estimates expressed in $\log_{10}$ NAT-detectable units/mL, after correcting for the equivalent volume of the test sample.

Relative Potencies

For quantitative assays, potencies of samples 2, 3, and 4 were estimated relative to sample 1 by using parallel-line analysis of log-transformed data. For qualitative assays, relative potencies were determined by using parallel-line analysis of probit-transformed data. Statistical analyses were performed by using SAS/STAT version 9.3 (SAS Institute, Cary, NC, USA). Estimation of endpoint dilution and relative potencies was performed by using CombiStats version 4.0 (European Directorate for the Quality of Medicines and HealthCare/Council of Europe, Strasbourg, France).

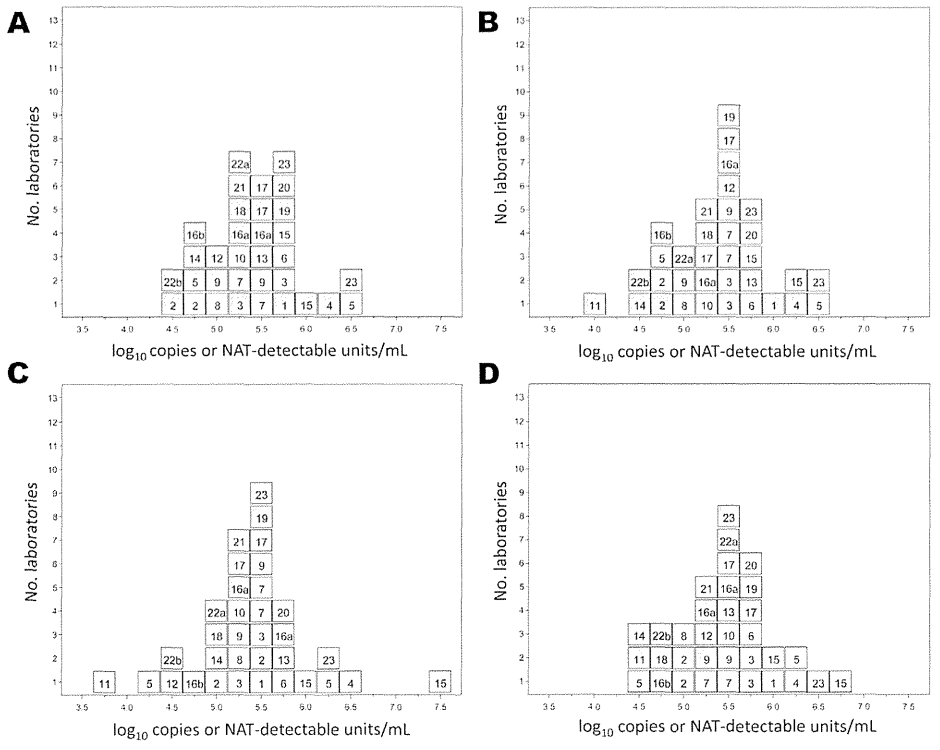

Figure 1. Histograms showing results for quantitative and qualitative assays conducted by 23 laboratories for the determination of the hepatitis E virus (HEV) RNA content of sample 1 (A), sample 2 (B), sample 3 (C), and sample 4 (D). White indicates quantitative assays ($\log_{10}$ copies/mL); gray indicates qualitative assays ($\log_{10}$ nucleic acid amplification technique (NAT)–detectable units/mL). Number of laboratories is indicated on the vertical axis. Laboratory code numbers are indicated in the respective boxes.

Results

Data were returned by 23 of the 24 participating laboratories; 20 sets of qualitative data and 14 sets of quantitative data were evaluated. The assays used by the participants are shown in online Technical Appendix Table 1 (wwwnc.cdc.gov/EID/article/19/5/12-1845-Techapp1.pdf). All assays were developed in-house and were either conventional or nested RT-PCRs or based on real-time RT-PCR.

Quantitative and Qualitative Assay Results

Laboratory mean estimates for quantitative assays (in $\log_{10}$ copies/mL) and qualitative assays (in NAT-detectable $\log_{10}$ units/mL) for the HEV preparations are shown in histogram form in Figure 1, which shows that laboratory means are more variable for the qualitative assays than the quantitative assays, reflecting different assay sensitivities and lack of standardization. The individual laboratory means are given in online Technical Appendix Tables 2 and 3; relative variation of the individual laboratory estimates for the quantitative assays is illustrated by the box-and-whisker plots in Figure 2. Intralaboratory variation was lower than the interlaboratory variation for both types of assays (data not shown).

Determination of Overall Laboratory Means

The means for all the laboratories performing quantitative assays are shown in Table 2. The means for sample 1 and sample 2, replicates for the candidate WHO IS, were 5.58 $\log_{10}$ and 5.60 $\log_{10}$ copies/mL HEV RNA, respectively, with good agreement between the replicate samples. The candidate Japanese national standard showed identical mean results of 5.66 $\log_{10}$ copies/mL HEV RNA for replicate samples 3 and 4.

The means for all the laboratories performing qualitative assays are also shown in Table 2; again, there was good agreement between the duplicate samples. Results for the qualitative assays showed 0.3-$\log_{10}$ lower mean estimates and a higher SD than those for the quantitative assays. The combined mean values for the replicate samples for both types of assays are shown in Table 2.

Relative Potencies

On the basis of the combined data from both qualitative and quantitative assays, the candidate WHO standard was determined to have a potency of 5.39 $\log_{10}$ units/mL (95% CI 5.15–5.63). This value was calculated with a combined endpoint evaluation of qualitative and quantitative data (restricted to dilutions in the range of 0.0 $\log_{10}$ to -2.5 $\log_{10}$) by means of a mixed linear model.

The potencies of samples 2, 3, and 4 were calculated relative to sample 1, taking the value of sample 1 as 5.39 $\log_{10}$ units/mL. The relative potencies for the quantitative and qualitative assays are shown in online Technical Appendix Tables 4 and 5, respectively. Table 3 summarizes the overall mean potencies relative to sample 1, with the 95% CIs, SDs, and geometric coefficients of variation. For the quantitative data from laboratory 9, no potency could be estimated by endpoint evaluation because only 1

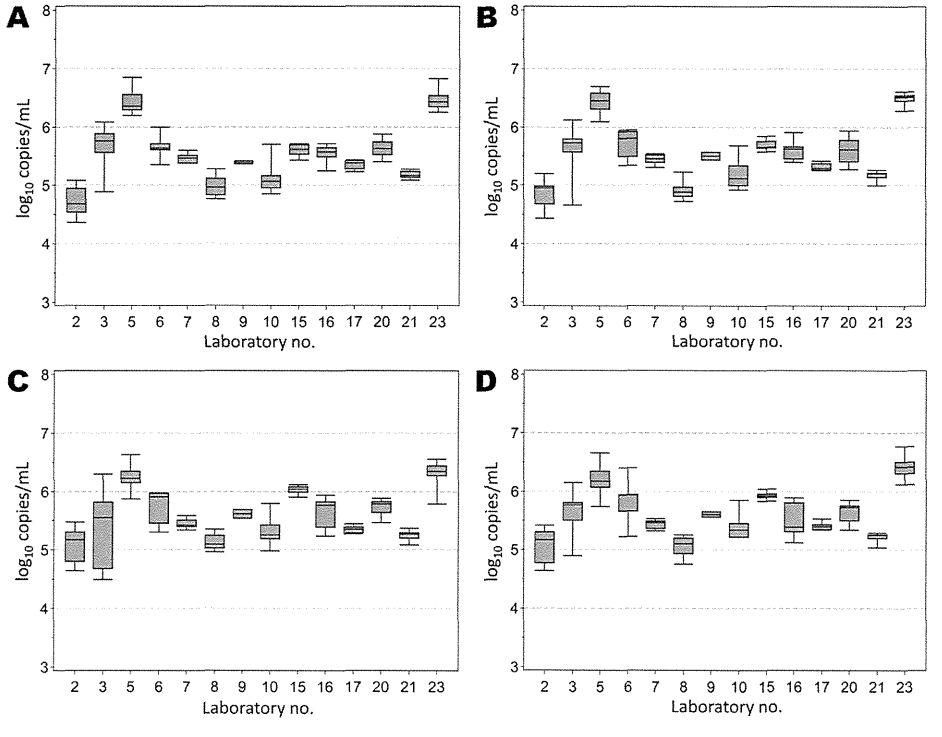

Figure 2. Box and whisker plots of the results for quantitative assays ($\log_{10}$ copies/mL) conducted by laboratories for the determination of the hepatitis E virus (HEV) RNA content of sample 1 (A), sample 2 (B), sample 3 (C), and sample 4 (D). Box indicates interquartile range; line within box indicates median; whiskers indicate minimum and maximum values observed. Laboratory code numbers are given on the horizontal axis.

Table 2. Overall mean estimates from quantitative and qualitative assays of HEV samples in study to establish a WHO International Standard for HEV RNA NAT-based assays*

Assay type and sample	No.	Mean (95% CI)†	SD	% CV
Quantitative				
1	123	5.58 (5.32–5.85)	0.54	98
2	125	5.60 (5.33–5.87)	0.53	94
1 + 2	248	5.59 (5.33–5.86)	0.55	99
3	124	5.66 (5.40–5.93)	0.45	77
4	125	5.66 (5.40–5.93)	0.44	76
3 + 4	249	5.66 (5.40–5.93)	0.44	76
Qualitative				
1	19	5.25 (5.01–5.50)	0.51	150
2	20	5.26 (4.97–5.56)	0.62	179
1 + 2	39	5.26 (5.08–5.44)	0.56	163
3	20	5.27 (4.90–5.64)	0.79	226
4	20	5.31 (5.02–5.61)	0.64	183
3 + 4	40	5.29 (5.07–5.52)	0.71	202

*Samples 1 and 2, replicate samples of the candidate WHO International Standard; samples 3 and 4, replicate samples of the candidate Japanese national standard. HEV, hepatitis E virus; WHO, World Health Organization; NAT, nucleic acid amplification technique; no., no. dilutions analyzed (in linear range for quantitative assays); % CV, geometric coefficient of variation.
†Values are $\log_{10}$ copies/mL for quantitative and $\log_{10}$ NAT–detectable units/mL for qualitative assays.

dilution was tested for each sample. The data are plotted in histogram form in Figure 3.

The data demonstrate that expressing the results as potencies relative to sample 1 (set as a standard with an assumed unitage of 5.39 $\log_{10}$ units/mL) results in a marked improvement in the agreement between the majority of methods and laboratories, as evidenced by the reduction in SDs. Furthermore, these data provide some evidence for commutability of the candidate standard for evaluation of HEV from infected persons, because samples 1 and 2 represent a different strain of HEV compared with samples 3 and 4.

Discussion

In this study, a wide range of quantitative and qualitative assays were used to determine the suitability and evaluate the HEV RNA content of the candidate standards. Although the methods used by the study participants were all developed in-house, most assays consistently detected the 2 HEV strains. On the basis of data from the qualitative and quantitative assays, the candidate WHO IS was estimated to have a potency of 5.39 $\log_{10}$ units/mL. For practical purposes, the candidate IS was assigned a unitage of 250,000 International Units (IU)/mL; because the difference in the overall mean for the candidate Japanese national standard was negligible compared with the WHO preparation, the 2 materials were assigned the same value. In the case of the quantitative assays, laboratories reported values in HEV RNA copies/mL. The participating laboratories used plasmid DNA containing HEV sequences, synthetic oligonucleotides, and in vitro–transcribed HEV RNA to control for copy number. In some cases, laboratories used HEV-containing plasma that had been calibrated against in vitro–transcribed HEV RNA. One laboratory prepared a standard by using stool-derived virus, the titer of which was determined by endpoint dilution and analysis by Poisson distribution. No standard method or common quantitation standard material was used; this fact is reflected in the variation observed for the quantitative results (in the order of 2 $\log_{10}$), which were improved by expressing the results against sample 1 as a common standard. For qualitative assays, the variation in NAT-detectable units was $\geq$3 $\log_{10}$, and as with quantitative assays, expressing potencies relative to sample 1 improved the agreement among the different laboratories and methods.

Many of the laboratories participating in the study used a real time RT-PCR developed in 2006 (*34*) that was designed to detect the 4 main genotypes of HEV. However, a recent study in the United Kingdom found a polymorphism in the probe-binding site in several HEV-infected patients who initially had negative test results using this assay (*40*). A modification of the probe, increasing the melting temperature, restored detection of the polymorphic virus strains. We identified a further polymorphism in an HEV strain (GenBank accession no. JN995566) from a plasma donor (*18*), located in the probe-binding site of the same assay; use of the modified probe improved the amplification curve for this virus strain (S. Baylis and T. Gärtner, unpub. data). Genetic variation and its potential effects on HEV RNA detection highlight the importance of confirmatory tests of different design, rather than reliance on single methods.

The WHO IS will be valuable for development of secondary standards traceable to the IU, which will facilitate comparison of results between laboratories and

Table 3. Overall mean potencies of samples 2, 3, and 4 relative to sample 1 from quantitative and qualitative analysis of HEV samples in study to establish a WHO International Standard for HEV RNA NAT-based assays*

Sample and assay type	No.	Mean (95% CI)†	SD	% CV
Sample 2				
Quantitative	19	5.46 (5.35–5.58)	0.23	3
Qualitative	13	5.42 (5.38–5.46)	0.07	1
Combined	32	5.45 (5.38–5.51)	0.18	2
Sample 3				
Quantitative	20	5.45 (5.27–5.65)	0.43	5
Qualitative	13	5.48 (5.37–5.59)	0.18	2
Combined	33	5.46 (5.35–5.58)	0.35	4
Sample 4				
Quantitative	20	5.51 (5.38–5.64)	0.29	3
Qualitative	13	5.47 (5.36–5.59)	0.19	2
Combined	33	5.49 (5.41–5.58)	0.25	3

*Mean potency values were determined by assigning a value of 5.39 $\log_{10}$ units/mL for sample 1. Samples 1 and 2, replicate samples of the candidate WHO International Standard; samples 3 and 4, replicate samples of the candidate Japanese national standard. HEV, hepatitis E virus; WHO, World Health Organization; NAT, nucleic acid amplification technique; no., no. dilutions analyzed (in linear range for quantitative assays); % CV, geometric coefficient of variation.
†Values are $\log_{10}$ copies/mL for quantitative and $\log_{10}$ NAT technique–detectable units/mL for qualitative assays.

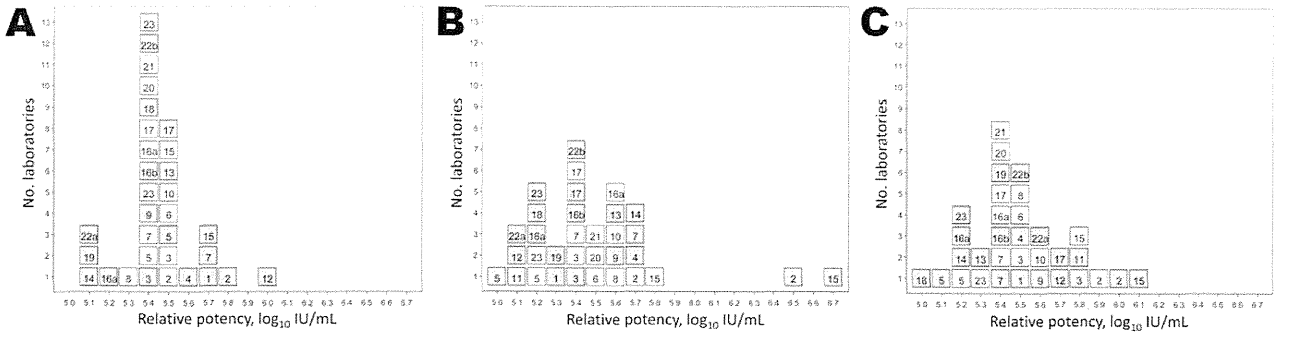

Figure 3. Histograms showing potencies of sample 2 (A), sample 3 (B), and sample 4 (C) compared with sample 1, the candidate World Health Organization International Standard for hepatitis E virus RNA for nucleic acid amplification technique (NAT)–based assays. White indicates quantitative assays (log_{10} copies/mL); gray indicates qualitative assays (log_{10} NAT–detectable units/mL). Number of laboratories is indicated on the vertical axis. Laboratory code numbers are indicated in the respective boxes.

determination of assay sensitivities and be helpful for validation purposes. We anticipate that the IS will find application in clinical laboratories, particularly in hepatitis reference laboratories that perform diagnosis and monitor HEV viral loads in chronically infected patients. The IS will also be helpful for research laboratories and blood and plasma centers that implement HEV NAT screening, regulatory agencies and organizations that are working to develop HEV vaccines, and manufacturers of HEV diagnostic kits.

The established WHO IS has been prepared by using a genotype 3a HEV strain. WHO has further endorsed a proposal by the PEI to prepare a genotype panel for HEV for NAT-based assays to continue standardization efforts for detection of this emerging infection. It is intended that the panel will contain representative strains of the 4 main genotypes of HEV that infect humans and notable subgenotypes. A new collaborative study will evaluate the IS against other genotypes and subgenotypes of HEV and investigate the commutability of the IS for standardization of assays for different genotypes of HEV. Laboratories that are able to provide high-titer HEV samples to aid in development of the proposed panel are requested to contact the authors.

In summary, WHO has established a genotype 3a HEV strain as the IS for HEV RNA (code number 6329/10), with an assigned a unitage of 250,000 IU/mL. The WHO IS for HEV RNA is available from PEI (www.pei.de).

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Effects of riboflavin and ultraviolet light treatment on platelet thrombus formation on collagen via integrin αIIbβ3 activation

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BACKGROUND: The adoption of pathogen reduction technology (PRT) is considered for the implementation of safer platelet (PLT) transfusion. However, the effects of PRT treatment on PLT thrombus formation under blood flow have not yet been fully clarified.

STUDY DESIGN AND METHODS: Leukoreduced PLT concentrates (PCs) obtained by plateletpheresis were treated with riboflavin and ultraviolet light (Mirasol PRT). PC samples were passed through a column filled with collagen-coated beads at a fixed shear rate after 1, 3, and 5 days of storage. The thrombus formation ability was evaluated by measuring collagen column retention rate. The change in the activation state of integrin αIIbβ3 on PLTs during storage was examined by flow cytometry.

RESULTS: The retention rate of the PRT-treated PLTs was significantly higher than that of the control PLTs on the day of treatment and decreased with storage but remained higher than those of the control during storage. This modification did not correlate with the total αIIbβ3 or fibrinogen binding on the PLTs but correlated significantly with PAC-1 binding. Mn^{2+}-induced αIIbβ3 activation also fully restored the retention rate in the Day 5 PRT-treated PLTs along with the increase in PAC-1 binding.

CONCLUSION: Riboflavin-based PRT treatment of PCs leads to the enhancement of thrombus formation on collagen, which is related to the activation status of αIIbβ3, which does not bind to fibrinogen but binds to PAC-1. The impact of this finding on the hemostatic or even thrombogenic potential in vivo must await clinical evaluation.

The safety of blood transfusion has been improved markedly owing to advances in blood donor selection and blood testing. Concerns still exist, however, because of a relatively high incidence of bacterial contamination of platelet (PLT) products and the possible risk of transfusion-transmitted infection by emerging pathogens.[1] Therefore, the adoption of pathogen reduction technologies (PRTs) that broadly and nonspecifically inactivate pathogens is being considered in many countries for the implementation of safer PLT transfusions. Current PRTs are based on inactivation methods using ultraviolet (UV) light irradiation with or without a photosensitizing agent against pathogen nucleic acids.[2] The Mirasol PRT system (Terumo BCT, Tokyo, Japan) uses UV light (265-370 nm) and a photosensitizing agent, riboflavin.[3] It is currently approved in some European countries for the treatment of PLT concentrates (PCs) in plasma or additive solution.[4]

Although PRT treatment enhances the safety of PCs, there are concerns about the decrease in product quality.[5] In vitro quality measurements of PCs during the storage period revealed a contribution of the PRT treatment to the PLT storage lesion when compared with the untreated controls. A decrease in pH, increase in P-selectin expression level, and decline in swirling were observed in PRT-treated PLTs.[6] In addition, the enhancement of glucose

ABBREVIATIONS: AP = apheresis; PC(s) = platelet concentrate(s); PRP = platelet-rich plasma; PRT(s) = pathogen reduction technology(-ies); TRAP = thrombin receptor–activating peptide.

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consumption and lactate production and the presence of mitochondrial dysregulation in PRT-treated PLTs were reported.[7,8] There have been various reports on the alteration of PLT functions caused by PRT treatment. The PLT aggregation in response to collagen and thrombin receptor–activating peptide (TRAP) was reported to be lesser in PRT-treated PLTs than in untreated control PLTs.[9] On the other hand, the adhesion of PRT-treated PLTs to assay plates was shown to be greater than that of control PLTs by the results of thrombus formation assay using Impact-R (DiaMed, Cressier sur Morat, Switzerland), which reflects the PLT ability for adhesion and aggregation associated with a shear stress caused by blood flow.[10] Moreover, using a perfusion model with rabbit aorta segments, the thrombus formation after storage was better maintained in PRT-treated PLTs than in control PLTs.[11] It seems that results in regard to the effects of PRT treatment on PLT functions vary depending on the assay methods used. In single-blind crossover studies, in vivo recovery and survival of PRT-treated PCs were reduced compared with those of untreated control units, as evidenced by reduced corrected count increment.[12,13] However, the results of a recent pilot study of PLT function in PRT-treated PLTs isolated from the circulation of patients after transfusion have suggested that these circulating cells may elicit hemostatic responses comparable to those in untreated PLTs.[14] To develop strategies that minimize the negative effects of PRT, it is necessary to determine the mechanisms by which PLTs are damaged.

In this study, we prepared columns filled with collagen-coated beads and performed a thrombus formation assay to clarify the effects of PRT treatment on PLT functions. In our collagen column method, thrombus formation on the solid-phase collagen similar to that observed in the damaged vascular subendothelium was assayed at a constant shear rate. This method better reflects physiologic conditions than PLT aggregometry, in which PLTs are simply stirred in the presence of agonists. Moreover, the collagen column method utilizes PLT-rich plasma (PRP), which is different from other methods using a flow chamber or Impact-R that require reconstituted blood or whole blood samples. Using the collagen column method, we determined the effects of PRT treatment on PLT thrombus formation and examined the mechanism underlying the effects.

MATERIALS AND METHODS

Materials

Common chemicals were purchased from Sigma-Aldrich (St Louis, MO) or Wako Pure Chemicals (Osaka, Japan). Copolymer plastic beads (165 μm in diameter on average) coated with porcine Type I collagen were purchased from ISK (Tokyo, Japan). A monoclonal Alexa Fluor 488–conjugated CD41 antibody (Clone MEM-06) was purchased from EXBIO (Prague, Czech Republic). A monoclonal fluorescein isothiocyanate (FITC)-conjugated PAC-1 antibody was purchased from BD Biosciences (San Jose, CA). A monoclonal phycoerythrin (PE)-cyanin 5.1 (PC5)–conjugated anti-CD41 antibody (Clone P2) was purchased from Beckman Coulter (Fullerton, CA). Polyclonal FITC-conjugated anti-human fibrinogen antibodies were purchased from Binding Site (PF056, Birmingham, UK) and Agrisera (IMS09-038-335, Vannas, Sweden).

Preparation of PCs

Informed consent was obtained from all healthy volunteers before apheresis (AP) donation. Leukoreduced AP-PCs (volume of 230 ± 16 mL and yield of $3.4 \times 10^{11} \pm 0.8 \times 10^{11}$ PLTs/bag) were collected using automated blood collection systems (Trima Accel, Terumo BCT; or CCS, Haemonetics, Braintree, MA).

PRT treatment

The Mirasol PRT treatment (n = 32, blood group composition: A, 12; B, 3; O, 17) was performed on Day 1 postcollection as described elsewhere.[6,8,15] Briefly, after the addition of 35 mL of riboflavin solution to an AP-PC bag at a final concentration of 50 μmol/L, the bag was exposed to UV light at a dose of 6.24 J/mL (265-370 nm) and allowed to stand for 30 minutes before placement on a flatbed agitator running at 55 agitations per minute. To the untreated control bag (n = 17, blood group composition: A, 4; B, 1; O, 12), 35 mL of a 0.9% saline solution was added. All PCs were kept at 20 to 24°C on the flatbed agitator for 5 days. All PLT samples were taken from bags, under sterile conditions, at 2 hours after PRT treatment and after 3 and 5 days of storage.

Thrombus formation assay and fluorescence microscopy

Collagen columns were prepared by filling silicon tubes (4-mm outer diameter, 2-mm inner diameter, and 50-mm length) with copolymer plastic beads coated with porcine Type I collagen using dry air (9 mL/min, 10 min) from an air compressor. Thus-prepared columns were set in an incubator at 37°C. PRP (30×10^{10} PLTs/L) prepared from PCs was incubated at 37°C for 10 minutes, and 0.5 mL of PRP was then passed through the columns at a shear rate of 750 per second by aspirating with a syringe pump (Fig. 1). This shear rate was calculated on the basis of previous reports.[16,17]

Retention rates obtained from PLT counts before and after the passage of PRP through the columns were used as indices of thrombus formation. All of the PRP samples before and after passage through the columns were

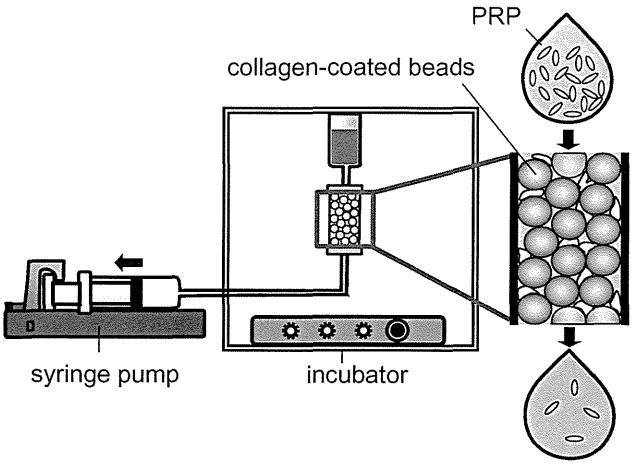

Fig. 1. Schematic diagram of collagen column system used for thrombus formation assay.

collected into plastic tubes containing ethylenediaminetetraacetic acid (5 mmol/L), and PLT count was determined using an automatic blood cell counter (XS-800i, Sysmex, Kobe, Japan). PLT retention rate (%) was calculated as follows: [(total PLT count before passing through the column) – (total PLT count after passing through the column)]/(total PLT count before passing through the column) × 100.

In one experiment, beads were taken from the columns through which PRP was passed and fixed with 1% paraformaldehyde phosphate-buffered saline (PBS) for 1 hour. The beads were then fluorescently stained with an Alexa Fluor 488–conjugated anti-CD41. The fluorescent images of PLT thrombus formed on the beads were observed by fluorescence microscopy (IX-71, Olympus, Tokyo, Japan) at 40× magnification.

Flow cytometry

Total αIIbβ3 level was measured on the basis of the level of binding of the anti-CD41 (P2) to the αIIbβ3 complex. The state of αIIbβ3 activation was evaluated on the basis of PAC-1 binding or fibrinogen binding. PLT samples were incubated with an FITC-conjugated PAC-1 antibody or FITC-conjugated polyclonal anti-human fibrinogen antibodies and a PE-cyanin 5.1–conjugated anti-CD41 for 20 minutes at room temperature. Isotype control antibodies were also included as controls. The samples were fixed with 1% paraformaldehyde PBS and analyzed by flow cytometry using the accompanying software (Cytomics FC500 and CXP software Version 2, respectively, Beckman Coulter, Miami, FL). Fluorescence data from 10,000 PLT events were collected in the logarithmic mode.

To examine the maximum binding of fibrinogen to PLTs, PLT samples, either PRT treated or untreated, were stimulated with 100 μmol/L TRAP for 5 minutes and stained with an FITC-conjugated anti-fibrinogen antibody and then subjected to flow cytometry analysis.

Mn^{2+} treatment

To clarify the effect of αIIbβ3 activation on thrombus formation, in some experiments, PRP samples were incubated with 1 mmol/L $MnCl_2$ as the activating agent at 37°C for 5 minutes before the thrombus formation assay and flow cytometry. As previously shown by others, this concentration of Mn^{2+} can fully activate αIIbβ3.[18,19]

Statistical analysis

Results are presented as mean ± SD. The t test was conducted with p values of not more than 0.05 indicating a significant difference. Correlations were determined using Spearman's rank correlation method. A correlation coefficient of more than 0.75 between methods was considered to indicate a good to excellent relationship. A correlation coefficient from 0 to 0.25 indicated a poor or no relationship.[20]

RESULTS

Effects of PRT treatment on PLT thrombus formation

We measured retention rate as an index of thrombus formation by the collagen column method. The retention rate of the PRT-treated PLTs on the day of treatment (Day 1) was 97.3 ± 6.1%, which was significantly higher than that of the control PLTs, 39.5 ± 21.7%, by more than twofold (Fig. 2A). Riboflavin alone without UV radiation did not increase the retention rate (46.1 ± 23.3%, n = 7, data not shown). We also observed the fluorescent images of PLT thrombi formed on the collagen-coated beads taken from the columns through which PRP passed. There were more PLTs and larger thrombi that covered multiple beads through which the Day 1 PRT-treated PLTs passed than on the beads through which the control PLTs passed (Fig. 2B). The retention rate of the PRT-treated PLTs was highest on Day 1 and decreased with storage period. The retention rate of the PRT-treated PLTs was significantly higher than that of the control PLTs throughout the storage period (Fig. 2A). These findings showed that PRT treatment enhanced the thrombus formation of the PLTs on collagen and that the thrombus formation was well maintained during the storage period.

Effects of PRT treatment on αIIbβ3

αIIbβ3 molecules on the PLT surface play a crucial role in thrombus formation. We examined the effects of PRT treatment on αIIbβ3 using the P2 antibody, which

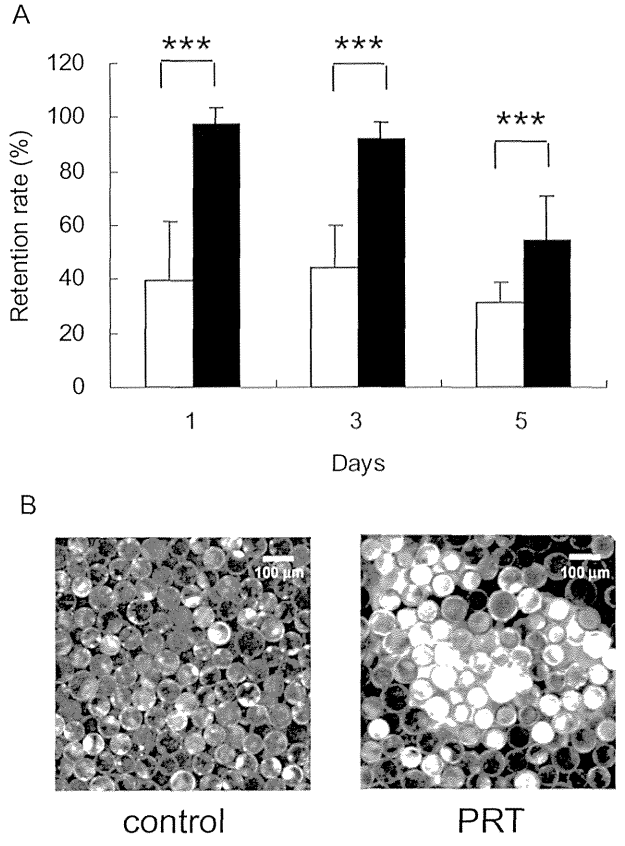

Fig. 2. Effects of PRT treatment on PLT thrombus formation. (A) Thrombus formation by collagen column method: control (□), PRT treated (■). (B) Typical fluorescent image of thrombus on collagen-coated beads through which Day 1 PLTs passed: (left) control; (right) PRT treated. Thrombus formation was determined on the basis of retention rate (%) presented as mean ± SD. n = 17 (□); n = 32 (■). ***p < 0.001.

recognizes the αIIbβ3 complex; the PAC-1 antibody, which recognizes conformational changes caused by αIIbβ3 activation; and the anti-fibrinogen antibody. There was no significant difference in the total αIIbβ3 level between the PRT-treated PLTs and the control PLTs on Day 1 (Fig. 3A). From the third day of storage, the total αIIbβ3 level on the PRT-treated PLTs was significantly higher than that on the control PLTs. The PAC-1 binding on the PRT-treated PLTs was significantly higher than that on the control PLTs (85.3 ± 9.4% vs. 10.7 ± 7.1%) on Day 1 (Fig. 3B) and decreased with storage time while maintaining a higher level than that on the control PLTs throughout the storage period. Using PF056, the level of fibrinogen binding on the PRT-treated PLTs (5.26 ± 1.34) was significantly higher than that on the control PLTs (4.08 ± 0.61) on Day 1 (Fig. 3C). However, it gradually increased with storage time compared with the control, which is in marked contrast to PAC-1 binding which shows a decreasing trend with storage time. The increasing trend of fibrinogen binding was confirmed with the other polyclonal antibodies specific for fibrinogen (data not shown). When the Day 1 PRT-treated PLTs were stimulated by TRAP, fibrinogen binding level markedly increased by 10.6- and 7.9-fold in terms of mean fluorescence intensity for control and PRT-treated PLTs, respectively (n = 4, data not shown), indicating that the fibrinogen binding is only partial although it increases with storage time. These findings show that PRT treatment immediately activated αIIbβ3 and sustained αIIbβ3 activation during the storage period; however, the sustained αIIbβ3 activation did not correspond to the increasing trend of total αIIbβ3 level and fibrinogen binding level.

Correlation between thrombus formation and activation of αIIbβ3

We compared the retention rate measured by the collagen column method with the activation marker measured by flow cytometry for all the PRT-treated and control PLT samples to determine the correlation between thrombus formation and αIIbβ3 activation (n = 49; Table 1). There were no correlations between retention rate and total αIIbβ3 level (P2 binding) or fibrinogen binding level (Spearman $r < 0.25$). However, there was a significant correlation between retention rate and αIIbβ3 activation (PAC-1 binding; Spearman $r = 0.840$, $p < 0.001$). These findings suggest that αIIbβ3 activation affected the thrombus formation as verified by the collagen column method, whereas the thrombus formation did not correlate with the total αIIbβ3 level or fibrinogen binding level.

Effects of Mn^{2+} on αIIbβ3 and thrombus formation

We measured the retention rate and PAC-1 binding level in the Day 5 PRT-treated PLTs and control PLTs after incubation with 1 mmol/L $MnCl_2$ at 37°C for 5 minutes to determine whether thrombus formation is restored by the reactivation of αIIbβ3. Mn^{2+} directly activates αIIbβ3 extracellularly without being mediated by signals from inside to outside the PLTs. The PAC-1 binding level in the PRT-treated PLTs significantly increased from 36.2 ± 19.2% to 95.6 ± 2.8% whereas that in the control PLTs also increased from 8.5 ± 6.5% to 66.9 ± 22.7% (Fig. 4A). The retention rate in the PRT-treated PLTs significantly increased from 54.3 ± 16.5% to 88.5 ± 15.6% whereas that in the control PLTs increased from 31.3 ± 7.7% to 82.5 ± 12.3% (Fig. 4B). As is the case for the Day 1 PRT-treated PLTs, there were many PLTs and large thrombi adhering to multiple beads through which the PRT-treated PLTs and control PLTs passed after the addition of Mn^{2+} (Fig. 4C). These findings suggest that thrombus formation on collagen was restored owing to the reactivation of αIIbβ3 in the PRT-treated PLTs after the addition of Mn^{2+}.

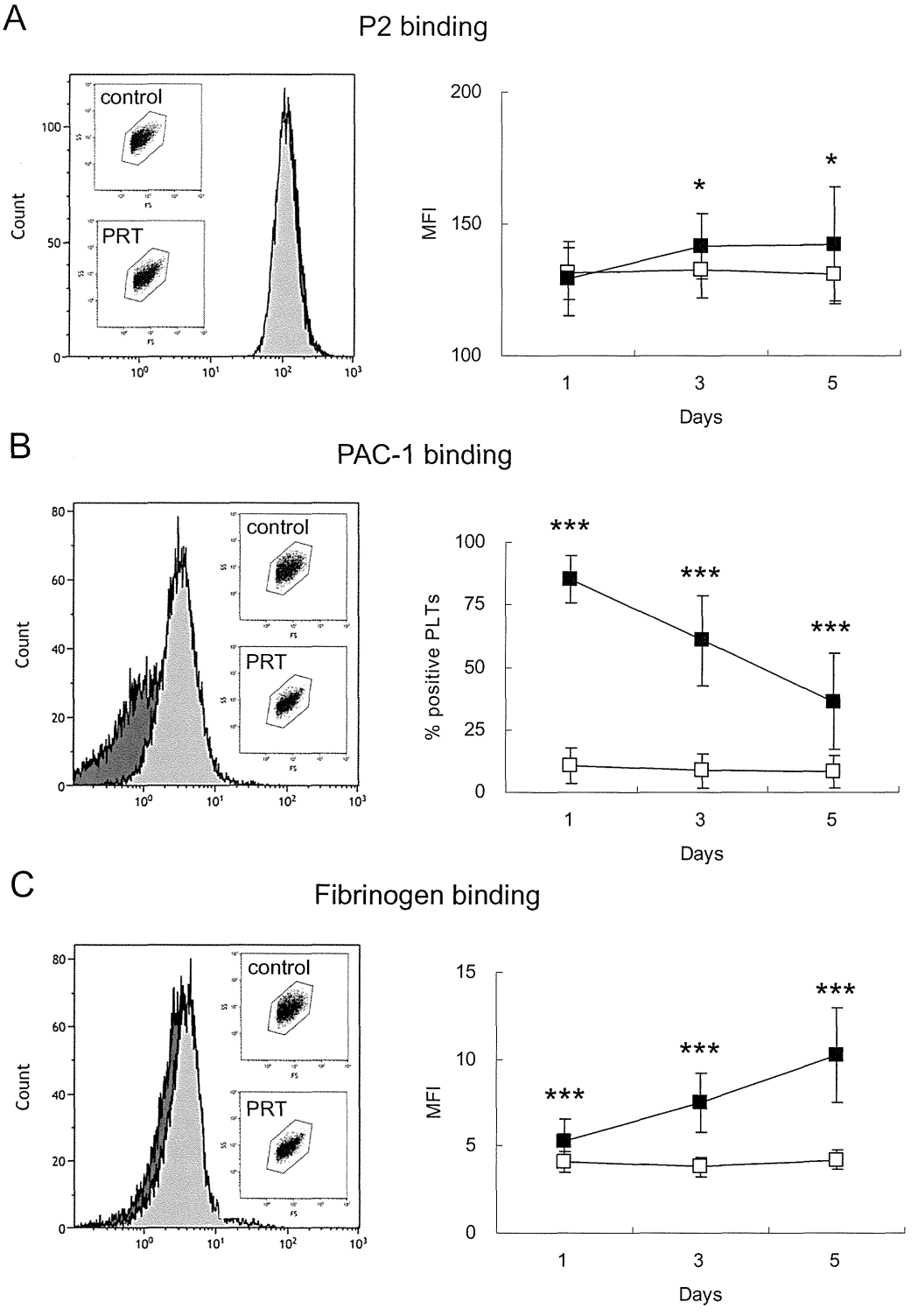

Fig. 3. Effects of PRT treatment on αIIbβ3 level and fibrinogen binding as determined by flow cytometry. (A) Total αIIbβ3 level represented as CD41 positivity; (B) activated αIIbβ3 represented as PAC-1 binding; (C) fibrinogen binding. Figure 3C shows representative results obtained using the fibrinogen-specific antibody PF056, but the same results were obtained using the other antibodies specific for fibrinogen (IMS09-038-355). Representative histograms (left column) indicate P2, PAC-1, and fibrinogen binding on the Day 1 control (■) and the Day 1 PRT-treated (□) PLTs. Boxes in each graph (right column) indicate the values for the control (□) and the PRT-treated (■) PLTs presented as mean ± SD. (Insets in histograms) Dot plots of forward scatter versus side scatter of a typical sample, with the gate set to exclude small vesicles and large aggregates. n = 17 (□); n = 32 (■). MFI = mean fluorescence intensity. *p < 0.05; ***p < 0.001.

TABLE 1. Correlation between retention rates and the flow cytometer variables

Variable	*r*	p value
P2 binding (MFI)	0.067	0.420
PAC-1 binding (% positive PLTs)	0.840	<0.001
Fibrinogen binding (MFI)	0.234	0.004

MFI = mean fluorescence intensity; *r* = Spearman's rank correlation coefficient.

DISCUSSION

With the aim of clarifying the effects of PRT treatment on thrombus formation in PLTs, we performed a thrombus formation assay using the collagen column method. This method was designed to assess PLT function in terms of thrombus formation under nearly physiologic conditions rather than turbidometric aggregometry, in which PLTs are stimulated by agonists under stirring condition. The collagen column method had also been used in a clinical setting for the evaluation of dose dependency of anti-PLT agent.[17] We found that thrombus formation in the PRT-treated PLTs was enhanced under flow in the collagen column. The enhanced thrombus formation was well sustained during the storage period. The finding of sustained thrombus formation after storage was in agreement with those of in vitro experiments using Impact-R and aorta segments. However, the thrombus formation ability was the highest immediately after the PRT treatment in the collagen column method, which was clearly different from those in the other two experiments. Using Impact-R, the surface coverage by aggregates of the PRT-treated PLTs on the day after PRT treatment was higher than that of the control PLTs, but there was no significant difference in the average size of aggregates between them.[10] In the experiments using rabbit aorta segments, the adhesive and cohesive functions of the PRT-treated PLTs were higher on Day 5 but lower on the day of PRT treatment compared with those of the control PLTs.[11] Thus, the evaluation of the ability of thrombus formation of PRT-treated PLTs may differ depending on the assay system used, and this awaits careful observation in clinical studies.

We considered that αIIbβ3 is involved in the enhancement of thrombus formation in the PRT-treated PLTs. The Mirasol PRT system uses UV radiation in a wide range of wavelengths from UV-A to UV-C (265 to 370 nm).[12,21] Verhaar and colleagues[22] reported that UV-C (254 nm) breaks disulfide bonds of αIIbβ3 and causes conformational changes of αIIbβ3, which leads to the enhancement of fibrinogen binding. Zhi and coworkers[23] suggested that UV-B (290 to 320 nm) also activates αIIbβ3 and enhances fibrinogen binding and that protein kinase C is involved in αIIbβ3 activation. Whereas they examined the effects of UV radiation alone, we showed in this study that the Mirasol PRT system, which uses a photosensitizer as well, also induced the marked activation of αIIbβ3 and enhanced fibrinogen binding. Interestingly, αIIbβ3 activated by PRT treatment did not bind fibrinogen completely immediately after the treatment. Although the level of αIIbβ3 activation induced by PRT treatment decreased with storage time, the level of fibrinogen binding on PLTs slightly increased, indicating that the activation state of αIIbβ3 represented as PAC-1 binding did not correlate with fibrinogen binding.

We speculate that, although slight conformational changes recognized by the PAC-1 antibody occurred, αIIbβ3 activation induced by PRT treatment did not cause sufficient conformational changes for αIIbβ3 to bind fibrinogen, which presents a distinct status from αIIbβ3 activation induced by physiologic agonists such as thrombin.[24] Takagi and colleagues[25] reported that integrins exist in at least three conformational states depending on the activation level: a bent conformer, an extended conformer with a closed headpiece, and an extended conformer with an open headpiece. Bunch[26] suggested that the conformational change of αIIbβ3 needs an increase in both affinity and avidity to strongly bind fibrinogen. It is thus possible that αIIbβ3 activation induced by PRT treatment is only partial so that a proportion of αIIbβ3 molecules on the PLT surface may bind fibrinogen or those molecules may weakly bind fibrinogen. The reason why the level of fibrinogen binding onto αIIbβ3 increased during storage was unclear. There may be effects of intrinsic activation of αIIbβ3 associated with the PLT storage lesion.

We found a strong correlation between thrombus formation assayed by the collagen column method and αIIbβ3 activation represented as PAC-1 binding. There was no correlation between thrombus formation and total αIIbβ3 level represented as CD41 positivity or fibrinogen binding. It is thus possible that the conformational changes of αIIbβ3 caused by PRT treatment enhanced the thrombus formation on collagen-coated beads. Jackson[27] reported that the contact of PLTs with collagen or the von Willebrand factor under various flow conditions activates αIIbβ3 and promotes thrombus growth. It seems that αIIbβ3 rapidly shifts from being partially activated to fully activated when the PRT-treated PLTs come into contact under flow with the collagen on the beads, leading to the enhanced thrombus formation. The thrombus formation assay by the collagen column method seemed to reflect the activation state of αIIbβ3 in PRT-treated PLTs quite well.

Both the thrombus formation and the PAC-1 binding to αIIbβ3 on the PRT-treated PLTs were reduced on Day 5. When Mn^{2+} was added to the PLTs, the αIIbβ3 on the PRT-treated PLTs as well as that in the control PLTs was activated, resulting in the restoration of thrombus formation. Mn^{2+} directly and fully activates αIIbβ3 without being mediated by inside-out signals from within the PLTs and enables the binding of αIIbβ3 to fibrinogen and

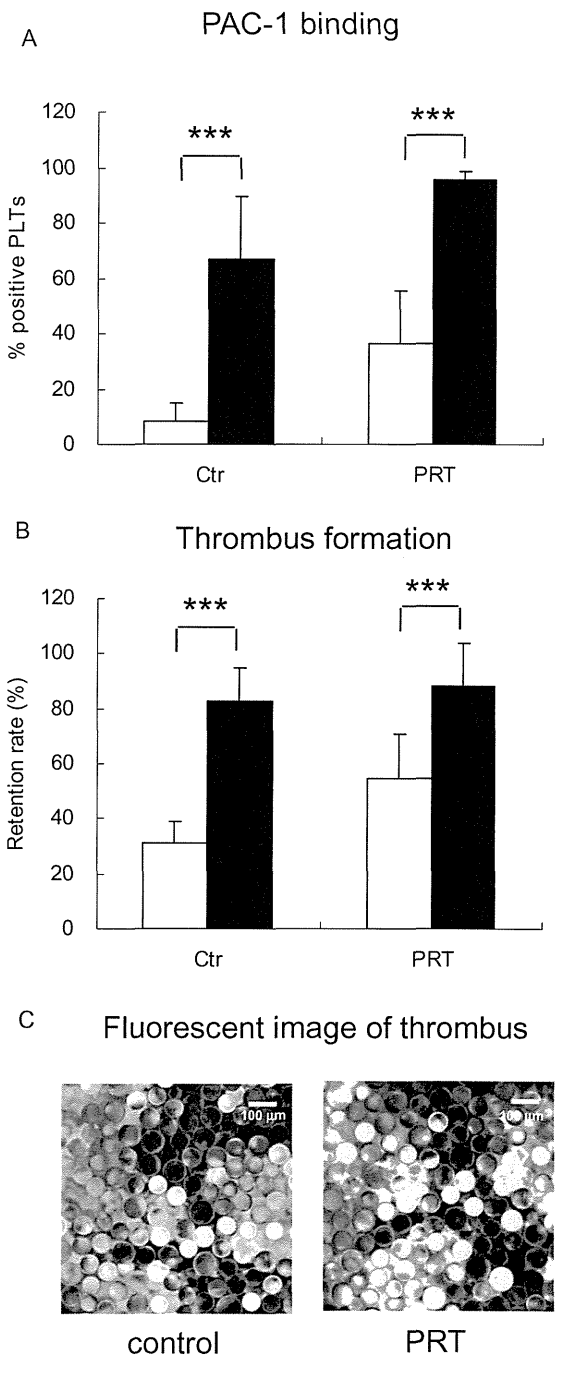

Fig. 4. Effects of Mn^{2+} on αIIbβ3 activation and thrombus formation. (A) Activated αIIbβ3 represented as PAC-1 binding on Day 5 control PLTs (Ctr, n = 7) and Day 5 PRT-treated PLTs (PRT, n = 24) in the presence (■) or absence (□) of Mn^{2+}. (B) Enhancement of thrombus formation determined by collagen column method in Day 5 control PLTs (Ctr, n = 7) and Day 5 PRT-treated PLTs (PRT, n = 24) in the presence (■) or absence (□) of Mn^{2+}. (C) Typical fluorescent image of thrombus on collagen-coated beads through which Day 5 PLTs incubated with Mn^{2+} passed: (left) control, (right) PRT-treated. Thrombus formation was determined on the basis of retention rate (%) presented as mean ± SD. ***p < 0.001.

fibrin.[19,28,29] The result showed a sufficient potential of reactivation of Day 5 PRT-treated PLTs in terms of αIIbβ3 activation and the restoration of thrombus formation in the column, suggesting that the functions of αIIbβ3 are not impaired. After immediate activation by PRT treatment, the αIIbβ3 in the PRT-treated PLTs gradually returned to the status with lower-level activation during storage. However, it was intriguing that the activation state lasted as long as 5 days with decreasing trend that seemed reversible.[30,31] Although we could not find an explanation for this reversible and long-lasting αIIbβ3 activation, it may be related to the activation state of αIIbβ3 that did not bind fibrinogen.[26,32]

In this study, thrombus formation ability was measured by the collagen column method as an index of PLT function. In the collagen column method, however, only the reaction between PLTs and collagen, the major component of the subendothelium, was evaluated in the absence of RBCs. The reaction between PLTs and collagen is important for evaluating the PLT function in the initial phase of thrombus formation. However, how much this reaction contributes to the complicated in vivo thrombus formation process has not been fully clarified yet. In addition, how the insufficient αIIbβ3 activation in PRT-treated PLTs leads to the enhancement of thrombus formation on collagen is unclear in our study. However, it has been reported that the signaling from the collagen receptor regulates αIIbβ3 activation and the subsequent thrombus formation.[33] We consider that there is a close relationship between the enhancement of thrombus formation and insufficient αIIbβ3 activation.

Although our results show the increased and sustained capacity of binding of PRT-treated PLTs to collagen, they indicate a possibility of unwanted binding of treated PLTs to undamaged endothelial cells under physiologic conditions. Although we do not have any expectation on this issue with our current system, we are currently developing an aggregometric system that could evaluate PLT aggregation caused by shear stress in the absence of collagen.

In conclusion, our studies using the collagen column method indicated that PRT treatment enhanced the thrombus formation on collagen and that the ability of the thrombus formation was well maintained during the storage period. We consider that the sustained αIIbβ3 activation is related to the enhancement of thrombus formation. In the absence of the contact of PLTs with collagen, however, αIIbβ3 activation induced by PRT treatment may be only partial. Upon contact with collagen, thrombus is formed completely along with the reactivation of αIIbβ3. These results could have an implication in the clinical setting of PLT transfusion in that the modifications of surface glycoproteins on PLTs caused by PRT treatment might not significantly affect the hemostatic effects of PRT-treated PLTs when transfused. Ultimately, the clinical

impacts of these findings on the hemostatic or even thrombogenic potential of PRT-treated PLTs should be evaluated in appropriate clinical trials.

ACKNOWLEDGMENT

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CONFLICT OF INTEREST

The disposables and the instrumentation for conducting pathogen reduction by the Mirasol system were provided without charge by Terumo BCT. Otherwise, none of the authors of this article have any conflicts of interest to report as defined by AABB's policy.

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TOPICS トピックス

輸血用血液における病原体不活化技術の現状と新規技術の開発

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はじめに

輸血用血液は，問診に加えて，病原体の血清学検査法とウイルス遺伝子を高感度に検出できる核酸増幅法の導入によって感染症の発生頻度は急激に低下したものの，スクリーニング法の限界や検査が実施されていない病原体などの感染リスクが存在する．そのため，輸血の安全性を確保するための対策として，病原体の不活化法が検討されるようになった．現在のところ，血漿製剤と血小板製剤の病原体不活化法が実用化され，欧州の一部の国や地域で導入されている．しかし，輸血用血液で最も使用量が多い赤血球製剤では，実用化された方法はない．

本稿では，輸血用血液の不活化法の現状と問題点について述べる．

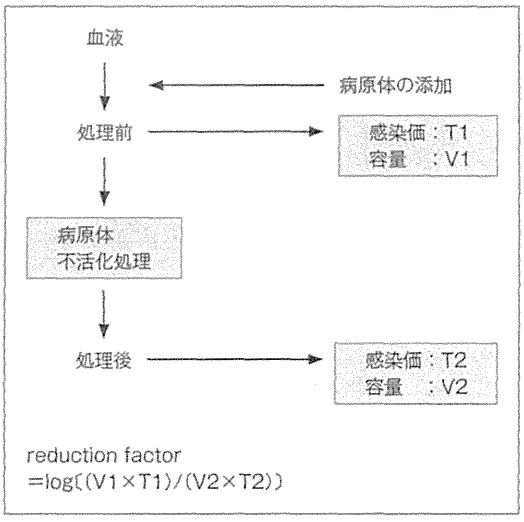

図1　病原体の不活化法の評価

病原体不活化法の評価法と効果について

不活化法を実施すれば，混入している病原体が全て不活化(感染力を失うこと)できるわけではない．不活化法にはそれぞれ不活化できる限界が存在する．不活化の能力を超えた量の病原体が混入していれば，感染価は減少するものの感染性を有した病原体が存在することになり，受血者の感染につながる．

日本では「不活化」という表現を使用するが，欧米では「低減化：リダクション」という言葉もよく使用されている．不活化効率は，図1に示すように，血液バッグに評価用の病原体を添加し，血液バッグに含まれる感染性を有する総病原体量(T1×V1)を計算する．次に不活化処理後の総病原体量(T2×V2)を計算し，リダクション値R=log(V1×T1/V2×T2)として求められる[1]．

ウイルスの場合，少量でも感染性ウイルスが

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表 1　血漿および血小板製剤の不活化法

方法	S/D 処理法	メチレンブルー法	アモトサレン法	リボフラビン法	(参考)検疫
対象製剤	血漿	血漿	血漿 血小板	血漿 血小板	血漿
製剤を構成する供血者数	多数	単	単	単	単
添加薬剤	界面活性剤	メチレンブルー	アモトサレン	リボフラビン	不要
照射	不要	可視光	紫外線 A 320～400 nm	紫外線 B 265～370 nm	不要
添加薬剤の除去	＋	＋	＋	不要	不要
使用実績	◎	◎	○	△	◎

残存していれば感染する可能性があるので，血液中のウイルス量が少ないウイルスに対しては効果的であるが，ウイルス量が多い感染症に対しては，処理能力の範囲までスクリーニング法などを併用して混入するウイルス量を減らさないと，不活化法単独で完全に病原体の感染を防ぐことは困難である．実際に不活化法を導入している血液センターにおいては，HBV(hepatitis B virus)，HCV(hepatitis C virus)，HIV(human immunodeficiency virus)の 3 つの血清学的に加えて核酸増幅法も併用している．不活化法の導入でこれら 3 つのウイルスのスクリーニング検査を止めた施設はない．

以上の記載だけでは，不活化法単独では利用価値がない技術と誤解されるかもしれないが，血小板の細菌感染予防では大いに効果を発揮している．これについては，「血小板の病原体不活化法」の項で後述する．

血漿の病原体不活化法

現在，実用化されている血漿の病原体不活化法には，S/D(solvent/detergent)処理法，メチレンブルー法，アモトサレン法，リボフラビン法の 4 つがある[2]．

S/D 処理法は欧州で主に導入され，数十 L～数百 L の血漿を混ぜ合わせてから，界面活性剤と変性剤を加えて病原体を処理する方法である(一般的に S/D プラズマと呼ばれている)．S/D 処理法は，エンベロープを有するウイルス(HIV・HBV・HCV など)に対しては極めて有用な病原体不活化法である．その一方で，エンベロープをもたないウイルス(A 型肝炎ウイルス・E 型肝炎ウイルス・パルボウイルス B19 など)に対しては全く効果はない．

メチレンブルー法やアモトサレン法，リボフラビン法は，個々のバッグに化学物質を添加し，それぞれ可視光，紫外線 A，紫外線 B を照射することによって病原体を不活化する方法である．S/D 処理法と異なり，混ぜ合わせることなく個々のバッグで不活化処理できる大きなメリットがある．しかも，エンベロープを有するウイルスだけでなく，エンベロープをもたないウイルスに対しても不活化することができる(ウイルスによって，抵抗性を有していて全く不活化できないものや，不活化効率が低いものもある)[3]．問題点には，添加する化学物質の毒性に懸念があることや，凝固因子などの活性がある程度失活し，低下することであることが挙げられる[2]．各不活化法の特徴を**表 1** に示す．